(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 July 2001 (12.07.2001)

PCT

(10) International Publication Number
WO 01/49691 A1

(51) International Patent Classification[7]: C07D 491/22, A61K 31/4741, 31/4745

(21) International Application Number: PCT/US00/15033

(22) International Filing Date: 14 June 2000 (14.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/474,099 29 December 1999 (29.12.1999) US

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(74) Agents: **CHINN, Richard, L.** et al.; Oblon, Spivak, McClelland, Maier & Neustadt, P.C., Crystal Square Five, Fourth Floor, 1755 Jefferson Davis Highway, Arlington, VA 22202 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

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(57) Abstract: β-Alanine esters of camptothecin compounds which are effective anti-tumor compounds are disclosed. These compounds inhibit the enzyme topoisomerase I and may alkylate DNA of the associated topoisomerase I-DNA cleavable complex.

TITLE OF THE INVENTION

CAMPTOTHECIN β-ALANINE ESTERS WITH TOPOISOMERASE I INHIBITION

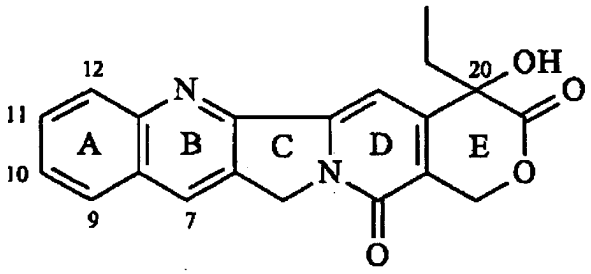
BACKGROUND OF THE INVENTION

Field of the Invention:

This invention relates to β-alanine esters of camptothecin compounds which inhibit the enzyme topoisomerase I and have anticancer activity. This invention is also related to the treatment of tumors in animals with camptothecin compounds.

Background of the Invention:

Camptothecin (CPT) is a naturally occurring cytotoxic alkaloid which is known to inhibit the enzyme topoisomerase I and is a potent anti-tumor agent. Camptothecin compounds have the general ring structure shown below.



Camptothecin was isolated from the wood and bark of *Camptotheca acuminata* by Wall et al. (Wall et al., 1966, J. Am. Chem. Soc., 88:3888).

Major synthetic efforts have been directed to derivatizing the A-ring and/or the B-ring to improve cytotoxic activity and to improve water-solubility.

The cytotoxic activity of camptothecin compounds is believed to arise from the ability of these compounds to inhibit both DNA and RNA synthesis and to cause reversible fragmentation of DNA in mammalian cells. Topoisomerase I relaxes both positively and negatively supercoiled DNA and has been implicated in various DNA transactions such as replication, transcription and recombination. The enzyme mechanism is believed to involve a transient breakage of one of the two DNA strands and the formation of a reversible covalent topoisomerase I enzyme-DNA complex. Camptothecin interferes with the DNA breakage-

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U.S. 4,894,456 describes methods of synthesizing camptothecin compounds which act as inhibitors of topoisomerase I and are effective in the treatment of leukemia (L-1210). U.S. 5,225,404 discloses methods of treating colon tumors with camptothecin compounds.

Numerous camptothecin compounds and their use as inhibitors of topoisomerase I are reported by U.S. 5,053,512; U.S. 4,981,968; U.S. 5,049,668; U.S. 5,106,742; U.S. 5,180,722; U.S. 5,244,903; U.S. 5,227,380; U.S. 5,122,606; U.S. 5,122,526; and U.S. 5,340,817.

U.S. 4,943,579 discloses the esterification of the hydroxyl group at the 20-position of camptothecin to form several prodrugs. This patent further discloses that the prodrugs are water soluble and are converted into the parent camptothecin compounds by hydrolysis.

Wall et al. U.S. 5,646,159 and 5,916,892 disclose C_{20} amino acid esters of CPT compounds.

Wall et al. U.S. 5,932,588 disclose CPT compounds bearing a C_7 methylene leaving group with C_{20} H or amino acid substitution.

Brangi et al., *Cancer Research*, 59, 5938-5946 December 1,1999, reports an investigation of Camptothecin resistance in cancer cells and reports the compound difluoro-10, 11-methylenedioxy-20(S)-camptothecin.

A need continues to exist, however, for camptothecin compounds having improved activity.

SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide camptothecin compounds having improved solubility and stability.

Another object of the present invention is to provide a method of treating leukemia or solid tumors in a mammal in need thereof by administration of a camptothecin compound.

Another object of the present invention is to provide a method of inhibiting the enzyme topoisomerase I and/or alkylating DNA of associated DNA-topoisomerase I by

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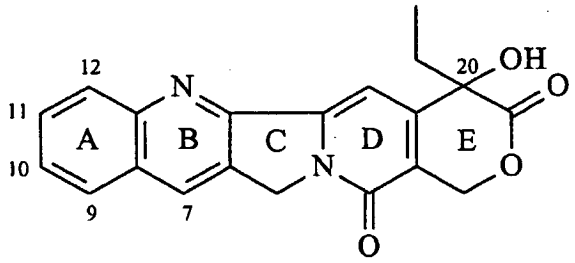

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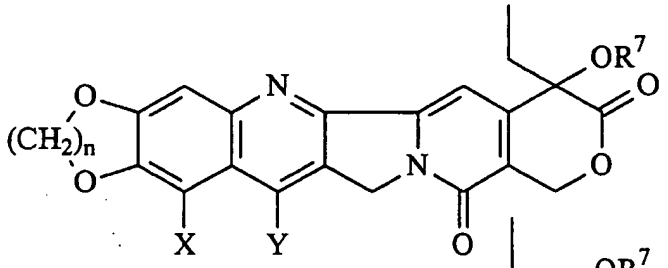
Another object of the present invention is to provide a method of inhibiting the enzyme topoisomerase I and/or alkylating DNA of associated DNA-topoisomerase I by

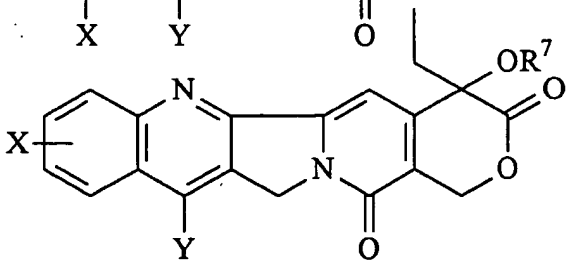
contacting a DNA-topoisomerase I complex with a camptothecin compound.

These and other objects of the present invention are made possible by amino acid esters of camptothecin compounds having the structure (I) or (II):



(I)

or



(II)

where

X and Y are each independently NO_2, NH_2, H, F, Cl, Br, I, COOH, OH, O-C_{1-6} alkyl, SH, S-C_{1-6} alkyl, CN, NH-C_{1-6} alkyl, N(C_{1-6} alkyl)$_2$, CHO, C_{1-8} alkyl, N_3,

-Z-$(CH_2)_a$-N-($(CH_2)_bOH)_2$, wherein Z is selected from the group consisting of O, NH and S, and a and b are each independently an integer of 2 or 3,

-Z-$(CH_2)_a$-N-(C_{1-6} alkyl)$_2$ wherein Z is selected from the group consisting of O, NH and S, and a is an integer of 2 or 3,

-CH_2-L, where L is halogen (F, Cl, Br, I), $^+N_2$, $^+(OR^1)_2$, $^+S(R^1)_2$, $^+N(R^1)_3$, $OC(O)R^1$, OSO_2R^1, OSO_2CF_3, $OSO_2C_4F_9$, C_{1-6} alkyl-C(=O)-, C_{4-18} aryl-C(=O)-, C_{1-6} alkyl-SO_2-, perfluoro C_{1-6}alkyl-SO_2- and C_{4-18} aryl-SO_2-, (where each R^1 independently is C_{1-6} alkyl, C_{4-18} aryl or C_{4-18} ArC_{1-6} alkyl); or

-$CH_2NR^2R^3$, where (a) R^2 and R^3 are, independently, hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-6} alkyl, C_{2-6} alkenyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy-C_{1-6} COR^4 where R^4 is hydrogen, C_{1-6} alkyl, perhalo-C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-6} alkyl, C_{2-6} alkenyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkoxy-C_{1-6} alkyl, or (b) R^2 and R^3 taken together with the nitrogen atom to which they are attached form a saturated 3-7 membered heterocyclic ring which may contain a O, S or NR^5 group, where R^5 is hydrogen, C_{1-6} alkyl, perhalo-C_{1-6} alkyl, aryl, aryl substituted with one or more groups selected from the group consisting of C_{1-6} alkyl, halogen, nitro, amino, C_{1-6} alkylamino, perhalo-C_{1-6} alkyl, hydroxy-C_{1-}

$_6$ alkyl, C_{1-6} alkoxy, C_{1-6} alkoxy-C_{1-6} alkyl and -COR^6 where R^6 is hydrogen, C_{1-6} alkyl perhalo-C_{1-6} alkyl, C_{1-6} alkoxy, aryl, and aryl substituted with one or more C_{1-6} alkyl, perhalo-C_{1-6} alkyl, hydroxy-C_{1-6} alkyl, or C_{1-6} alkoxy-C_{1-6} alkyl groups;

R^7 is C(O)-CH_2-CH_2-NR^8R^9, R^8 and R^9 are, independently, hydrogen, C_{1-8} alkyl, C(O)-$(CH_2)_m$-$NR^{10}R^{11}$, where m is an integer from 1 to 6, or -C(O)$CHR^{12}NR^{13}R^{14}$, where R^{12} is the side chain of one of the naturally occurring α-amino acids and R^{10}, R^{11}, R^{13} and R^{14} are each independently hydrogen or C_{1-8} alkyl; and

n is an integer of 1 or 2,

and salts thereof.

β-Alanine esters of camptothecin compounds are a potent inhibitor of topoisomerase I, and have greater stability and solubility than that of esters of naturally occurring amino acids.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless indicated to the contrary, the term "alkyl" as used herein means a straight-chain or branched chain alkyl group with 1-30, preferably 1-18 carbon atoms, more preferably 1-8 carbon atoms, including methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, n-hexyl, n-heptyl, n-octyl, 2-ethylhexyl, n-nonyl, n-decyl, undecyl, dodecyl, myristyl, heptadecyl and octadecyl groups. The term "alkyl" also includes C_{3-30} cycloalkyl groups such as cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl groups.

Unless indicated to the contrary, the term "aryl" as used herein means a carbocyclic aromatic ring having 6-18 carbon atoms, preferably 6-10 carbon atoms in the aromatic ring structure. The aromatic rings may be substituted by one or more alkyl group, preferably alkyl groups having 1-10 carbon atoms. A particularly preferred aryl group is phenyl.

Unless indicated to the contrary, the term "aralkyl" as used herein means a straight-chain or branched chain alkyl group as defined above for the term "alkyl" bonded to an aryl group as defined above for the term "aryl". Preferred aralkyl groups are benzyl, phenethyl, etc.

As used herein, the term "acyl" means formyloxy and acyl moieties derived from aromatic carboxylic acids, heterocyclic carboxylic acids, aralkyl carboxylic acids, as well as

alkyl and aromatic sulfonic acids. The alkyl groups of these acyloxy moieties may be a straight-chain or branched-chain alkyl group with 1-7 carbon atoms. Additionally, the acyl moiety may contain one or more unsaturated carbon-carbon bonds and may also carry one or more substituents such as halogen, amino and hydroxyl groups.

The camptothecin compounds of the present invention may bear a leaving group at one or more of the positions C_7 or C_9 of the camptothecin ring structure. More specifically, the leaving group is a group of the formula $-CH_2-L$, where L is a functional group which can be easily displaced, i.e. L is a good leaving group in nucleophilic substitution reactions. Suitable groups L include halogen (F, Cl, Br, I), $^+N_2$, $^+(OR^1)_2$, $^+S(R^1)_2$, $^+N(R^1)_3$, $OC(O)R^1$, OSO_2R^1, OSO_2CF_3, and $OSO_2C_4F_9$, C_{1-6} alkyl-C(=O)-, C_{4-18} aryl-C(=O)-, C_{1-6} alkyl-SO_2-, perfluoroC_{1-6} alkyl-SO_2- and C_{4-18} aryl-SO_2-,(where each R^1 independently is C_{1-6} alkyl, C_{4-18} aryl or C_{4-18} ArC_{1-6} alkyl).

While not being bound by any particular theory, it is believed that nucleophilic groups on DNA displace leaving group L from the camptothecin compounds of the present invention resulting in alkylation of the DNA by the alkylating group of the camptothecin ring structure. Suitable nucleophilic groups present in DNA include the nucleophilic groups found in DNA bases adenine, guanine, thymine, and cytosine, such as NH_2, -NH- and =N- groups. When a camptothecin compound of the invention having a $-CH_2-L$ group is contacted with DNA, nucleophilic displacement of leaving group L results in alkylation of the nucleic acid. The compounds of the present invention exhibit a novel anti-tumor activity by alkylating DNA.

Camptothecin compounds have an asymmetric carbon atom at the 20-position making two enantiomeric forms, i.e., the (R) and the (S) configurations, possible. This invention includes both enantiomeric forms and any combinations or mixtures of these forms. The invention also includes other forms of the camptothecin compounds including solvates, hydrates, polymorphs, salts, etc. Particularly preferred compounds are camptothecin derivatives having the (S) configuration at the 20-position.

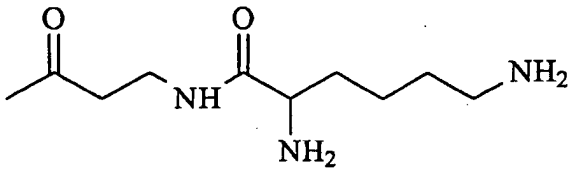
In a preferred embodiment, X is NO_2, NH_2, H, F, Cl, Br, I, COOH, OH, O-C_{1-6} alkyl, SH, S-C_{1-6} alkyl, CN, CH_2NH_2, NH-C_{1-6} alkyl, CH_2NHC_{1-6} alkyl, N(C_{1-6} alkyl)$_2$, $CH_2N(C_{1-6}$ alkyl)$_2$, O-$CH_2CH_2N(CH_2CH_2OH)_2$, NH-$CH_2CH_2N(CH_2CH_2OH)_2$, S-$CH_2CH_2N(CH_2CH_2OH)_2$, O-$CH_2CH_2CH_2N(CH_2CH_2OH)_2$, NH-$CH_2CH_2CH_2N(CH_2CH_2OH)_2$, S- $CH_2CH_2CH_2N(CH_2CH_2OH)_2$, O-$CH_2CH_2N(CH_2CH_2CH_2OH)_2$, NH-

$CH_2CH_2N(CH_2CH_2CH_2OH)_2$, S-$CH_2CH_2N(CH_2CH_2CH_2OH)_2$, O-$CH_2CH_2CH_2N(CH_2CH_2CH_2OH_2)_2$, NH-$CH_2CH_2CH_2N(CH_2CH_2CH_2OH_2)_2$, S-$CH_2CH_2CH_2N(CH_2CH_2CH_2OH_2)_2$, O-$CH_2CH_2N(C_{1-6}$ alkyl$)_2$, NH-$CH_2CH_2N(C_{1-6}$ alkyl$)_2$, S-$CH_2CH_2N(C_{1-6}$ alkyl$)_2$, O-$CH_2CH_2CH_2N(C_{1-6}$ alkyl$)_2$, NH-$CH_2CH_2CH_2N(C_{1-6}$ alkyl$)_2$, S-$CH_2CH_2CH_2N(C_{1-6}$ alkyl$)_2$, CHO, N_2, C_{1-8} alkyl, CH_2-L where L is halogen (F,Cl, Br, I), $^+N_2$, $^+(OR^1)_2$ (where each R^1 independently is alkyl, aryl or aralkyl as defined above), $^+S(R^1)_2$, $^+N(R^1)_3$, $OC(O)R^1$, OSO_2R^1, OSO_2CF_3, $OSO_2C_4F_9$, C_{1-6}alkyl-C(=O)-, C_{4-18}aryl-C(=O)-, C_{1-6}alkyl-SO_2-, perfluoro C_{1-6}alkyl-SO_2- and C_{4-18}aryl-SO_2-.

In a preferred embodiment Y is H, C_{1-8} alkyl, or $CH_2NR^2R^3$ where (a) R^2 and R^3 are, independently, hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-6} alkyl, C_{2-6} alkenyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy-C_{1-6} COR^4 where R^4 is hydrogen, C_{1-6} alkyl, perhalo-C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-6} alkyl, C_{2-6} alkenyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkoxy-C_{1-6} alkyl, or (b) R^2 and R^3 taken together with the nitrogen atom to which they are attached form a saturated 3-7 membered heterocyclic ring which may contain a O, S or NR^5 group, where R^5 is hydrogen, C_{1-6} alkyl, perhalo-C_{1-6} alkyl, aryl, aryl substituted with one or more groups selected from the group consisting of C_{1-6} alkyl, halogen, nitro, amino, C_{1-6} alkylamino, perhalo-C_{1-6} alkyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkoxy-C_{1-6} alkyl and -COR^6 where R^6 is hydrogen, C_{1-6} alkyl perhalo-C_{1-6} alkyl, C_{1-6} alkoxy, aryl, and aryl substituted with one or more C_{1-6} alkyl, perhalo-C_{1-6} alkyl, hydroxy-C_{1-6} alkyl, or C_{1-6} alkoxy-C_{1-6} alkyl groups.

The group R^7 is a β-alanine ester, or amino acid peptide thereof.

Suitable side chains R^8 and R^9 appearing on the group R^7 are the side chains of the amino acids glycine, α-alanine, β-alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, lysine, arginine, histidine, aspartate, glutamate, asparagine, glutamine, cysteine and methionine. Moreover, the group R^7, may comprise two amino acid units linked by a peptide linkage. In particular the group R^7 may comprise a β-alanine group linked to a lysine of the structure



Moreover, the group R^7 may provide basis for the formation of a mono or di salts, via the free amine groups, such as a hydrochloride or dihydrochloride.

A synthon for attaching such a group to a terminal hydroxyl group is described by Hudkins et al. *Bioorg. Med. Chem. Lett,* **8** (1998)1873-1876).

Particularly preferred esters are the peptide ester based on β-alanine-lysine. These esters are pro-drugs which are converted to the camptothecin compound by hydrolysis of the ester bond. The esters may be prepared by the method described in U.S. 4,943,579 which is incorporated herein by reference for a more complete description of the process of preparing the esters and for a description of suitable esters formed by the process. The esterification synthon may need to introduced in a protected form, such that the reaction of amine groups is inhibited, followed by removal of the protecting group. Such protecting groups are well known to those of ordinary skill in the art and are described by Hudkins et al. *Bioorg. Med. Chem. Lett,* **8** (1998)1873-1876).

Specific non-limiting examples include 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-ethyl 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-chloromethyl 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-bromomethyl 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-hydroxymethyl-10, 11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin, 9-nitro 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin, 9-amino 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin, 7-ethyl-9-nitro 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin and 7-ethyl-9-amino 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin.

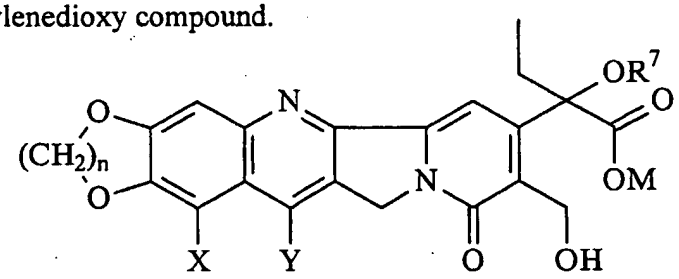
Additional specific non-limiting examples further include 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-ethyl 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-chloromethyl 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-bromomethyl 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-hydroxymethyl-10, 11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin, 9-nitro 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin, 9-amino 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin, 7-ethyl-9-nitro 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin and 7-ethyl-9-amino 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin.

Additional specific non-limiting examples include 20-O-β-ala-lys-20-(S)-camptothecin; 7-ethyl-20-O-β-ala-lys-20-(S)-camptothecin; 7-chloromethyl-20-O-β-ala-lys-

20-(S)-camptothecin; 7-bromomethyl-20-O-β-ala-lys-20-(S)-camptothecin;-20-O-β-ala-lys-20-(S)-camptothecin, 9-nitro-20-O-β-ala-lys-20-(S)-camptothecin, 9-amino-20-O-β-ala-lys-20-(S)-camptothecin, 7-ethyl-9-nitro-20-O-β-ala-lys-20-(S)-camptothecin and 7-ethyl-9-amino-20-O-β-ala-lys-20-(S)-camptothecin.

Additional specific non-limiting examples further include 20-O-β-ala-20-(S)-camptothecin; 7-ethyl-20-O-β-ala-20-(S)-camptothecin; 7-chloromethyl 20-O-β-ala-20-(S)-camptothecin; 7-bromomethyl-20-O-β-ala-20-(S)-camptothecin; 7-hydroxymethyl-20-O-β-ala-20-(S)-camptothecin, 9-nitro-20-O-β-ala-20-(S)-camptothecin, 9-amino-20-O-β-ala-20-(S)-camptothecin, 7-ethyl-9-nitro-20-O-β-ala-20-(S)-camptothecin and 7-ethyl-9-amino-20-O-β-ala-20-(S)-camptothecin.

Within the scope of the present invention, the lactone ring of the camptothecin compounds shown above may be opened by alkali metal or alkaline earth metal bases (MOH) for example, sodium hydroxide or calcium hydroxide to form alkali metal or alkaline earth metal salts of the open ring salt form of the camptothecin compounds, illustrated for example only for the alkylenedioxy compound.



Open ring compounds generally have better solubility in water. The group M may also be any pharmaceutically acceptable cation, obtained either directly by ring opening or by cation exchange of a ring open salt. Suitable groups M include Li^+, Na^+, K^+ and Mg^{+2}.

The compounds of the present invention may be prepared by conventional methods known to those of ordinary skill in the art, without undue experimentation.

The C_{20} OH CPT compounds of the present invention may be prepared by conventional methods known to those of ordinary skill in the art, such as that described by Wall et al. U.S. 5,122,526, the relevant portions of which are hereby incorporated by reference.

Substitution at the C_7 position may be conducted by condensation with the corresponding aldehyde of the C_7 substituent.

Esterification with an amino acid at C_{20} is possible by conventional methods known to those of ordinary skill in the art. Substitution at C_9 with groups such a nitro and amino is also possible in a manner analogous to that described in the literature.

The compounds of the invention having the group $-CH_2-L$ at C_9 are prepared from known 20(S)-CPT compounds bearing a halogen, for example, a bromine atom, at the C_9 position. The halogen atom can be readily converted into the corresponding cyano analog by reaction with CuCN, followed by hydrolysis to form the corresponding carboxy analog. The carboxy analog is reduced to the corresponding hydroxy methyl analog which can be reacted with Ph_3P-CCl_4 to provide the corresponding chloromethyl analog. The chloromethyl analog can be readily converted to the bromomethyl and iodomethyl analogs using LiBr or LiI. The remaining compounds of the invention are prepared from these compounds by reaction with the corresponding acid chloride, sulfonyl chloride, etc. These reactions are well known to one having ordinary skill in this art.

Compounds in which L is Br or I are readily prepared from the compound in which L is Cl by simple halide exchange employing LiBr or LiI in dimethylformamide (DMF) solution (Larock, R.C., Comprehensive Organic Transformations, VCH Publishers, Inc., p. 337, N.Y. 1989).

Alternatively, the 7-methyl compounds (L is H) can be prepared either by a Friedlander reaction employing the corresponding acetophenone, or by a free radical alkylation reaction (Sawada et al., 1991, Chem. Pharm. Bull., 39:2574). Free radical bromination of 7-methyl substrates can be accomplished by employing N-bromosuccinimide (NBS) in acetic acid (HOAc) under catalysis by benzoyl peroxide to give compounds in which L is Br.

Other compounds which possess oxygen-derived leaving groups, such as triflate or tosylate, are prepared from the 7-hydroxymethyl and/or 7-halomethyl compounds. The 7-hydroxymethyl compounds are prepared from the corresponding parent compounds by the hydroxymethylation reaction. (e.g. Sawada et al., 1991, Chem. Pharm. Bull., 39:2574) Treatment of these compounds with readily available sulfonic acid chlorides or anhydrides using known procedures (Stang et al., 1982, Synthesis, 85) provides the highly electrophilic substrates noted above. Alternatively, the compounds described above can be generated from any of the substrates where L is Cl, Br or I by reaction with the silver salt of the

corresponding acid (e.g., silver trifluoromethanesulfonate, silver tosylate, etc.) as described generally by Stang et al. and more specifically by Gramstad and Haszeldine (T. Gramstad and R.N. Haszeldine, 1956, J. Chem. Soc., 173).

C_{20} esters may be prepared by esterifying the 20-position hydroxyl group of a camptothecin compound to form an ester containing a water-soluble moiety. Generally, the camptothecin compound is initially suspended in methylene chloride or other inert solvent, stirred and cooled. To the cooled mixture is added one equivalent of an acid having the formula $HOOC-CH_2-CH_2-NR^8R^9$, where R^8 and R^9 are independently, hydrogen, C_{1-8} alkyl, $C(O)-(CH_2)_m-NR^{10}R^{11}$, where m is an integer from 1 to 6, or $-C(O)CHR^{12}NR^{13}R^{14}$, where R^{12} is the side chain of one of the naturally occurring α-amino acids and R^{10}, R^{11}, R^{13} and R^{14} are each independently hydrogen or C_{1-8} alkyl. Suitable side chains R^{12} are the side chains of the amino acids glycine, α-alanine, β-alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, leucine, arginine, histidine, aspartate, glutamate, asparagine, glutamine, cysteine and methionine. Particularly preferred esters are glycinate esters. One equivalent of dicyclohexylcarbodiimide (DCC) and a catalytic amount of an amine base, preferably a secondary or tertiary amine, are also added to the mixture, which is then stirred to complete the reaction. Any precipitate which forms is removed by filtration and the product is isolated after removal of the solvent.

The free amine(s) may be converted to an acid addition salt by the addition of a pharmaceutically acceptable acid. Suitable acids include both inorganic and organic acids. Suitable addition salts include, but are not limited to hydrochloride, sulfate, phosphate, diphosphate, hydrobromide, nitrate, acetate, malate, maleate, fumarate, tartrate, succinate, citrate, lactate, methanesulfonate, p-toluenesulfonate, palmoate, salicylate and stearate salts. The salts may be purified by crystallization from a suitable solvent.

The water-soluble 20-hydroxyl esters of the present invention are substantially less toxic than the parent compounds from which the esters are prepared.

The camptothecin compounds are administered in a dose which is effective to inhibit the growth of tumors. As used herein, an effective amount of the camptothecin compounds is intended to mean an amount of the compound that will inhibit the growth of tumors, that is, reduce the site of growing tumors relative to a control in which the tumor is not treated with the camptothecin compound. These effective amounts are generally from about 1-60 mg/kg

of body weight per week, preferably about 2-20 mg/kg per week.

The compounds of the present invention may be administered as a pharmaceutical composition containing the camptothecin compound and a pharmaceutically acceptable carrier or diluent. The active materials can also be mixed with other active materials which do not impair the desired action and/or supplement the desired action. The active materials according to the present invention can be administered by any route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

For the purposes of parenteral therapeutic administration, the active ingredient may be incorporated into a solution or suspension. The solutions or suspensions may also include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Another mode of administration of the compounds of this invention is oral. Oral compositions will generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the aforesaid compounds may be incorporated with excipients and used in the form of tablets, gelatine capsules, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like. Compositions may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents. Tablets containing the active ingredient in admixture with nontoxic pharmaceutically acceptable excipients which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by

known techniques to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed. Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

The tablets, pills, capsules, troches and the like may contain the following ingredients: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, corn starch and the like; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; and a sweetening agent such as sucrose or saccharin or flavoring agent such as peppermint, methyl salicylate, or orange flavoring may be added. When the dosage unit form is a capsule, it may contain, in addition to material of the above type, a liquid carrier such as a fatty oil. Other dosage unit forms may contain other various materials which modify the physical form of the dosage unit, for example, as coatings. Thus tablets or pills may be coated with sugar, shellac, or other enteric coating agents. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors. Materials used in preparing these various compositions should be pharmaceutically or veterinarially pure and non-toxic in the amounts used.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylethyl cellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethylene oxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (e.g., polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan mono-oleate). The aqueous suspension

may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, aspartame, saccharin, or sucralose.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oil suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water may be formulated from the active ingredients in admixture with a dispersing, suspending and/or wetting agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical composition of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan mono-oleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan mono-oleate. The emulsion may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, such as a solution of 1,3-butanediol. Among the

acceptable vehicles and solvents that may be employed are water and Ringer's solution, an isotonic sodium chloride. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables. Sterilization may be performed by conventional methods known to those of ordinary skill in the art such as by aseptic filtration, irradiation or terminal sterilization (e.g. autoclaving).

Aqueous formulations (i.e oil-in-water emulsions, syrups, elixers and injectable preparations) may be formulated to achieve the pH of optimum stability. The determination of the optimum pH may be performed by conventional methods known to those of ordinary skill in the art. Suitable buffers may also be used to maintain the pH of the formulation.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable nonirritating excipient which is solid at ordinary temperatures but liquid at the rectal temperatures and will therefore melt in the rectum to release the drug. Non-limiting examples of such materials are cocoa butter and polyethylene glycols.

They may also be administered by intranasal, intraocular, intravaginal, and intrarectal routes including suppositories, insufflation, powders and aerosol formulations.

The compounds of the present invention may also be administered in the form of liposome or microvesicle preparations. Liposomes are microvesicles which encapsulate a liquid within lipid or polymeric membranes. Liposomes and methods of preparing liposomes are known and are described, for example, in U.S. 4,452,747, U.S. 4,448,765, U.S. 4,837,028, U.S. 4,721,612, U.S. 4,594,241, U.S. 4,302,459 and U.S. 4,186,183. The disclosures of these U.S. patents are incorporated herein by reference. Suitable liposome preparations for use in the present invention are also described in WO-9318749-A1, J-02056431-A and EP-276783-A.

The camptothecin compounds may be used individually to inhibit the growth of tumors. Alternatively, combinations of two or more camptothecin compounds may be used or combinations of one or more camptothecin compounds with one or more known anti-tumor compounds. When a camptothecin compound is combined with a conventional anti-tumor compound, the camptothecin compound will generally be present in an amount ranging

from about 1-99 wt.%, preferably, 5-95 wt.% of the combined amount of camptothecin and conventional anti-tumor compound. The pharmaceutical compositions noted above may contain these combinations of compounds together with an acceptable carrier or diluent.

The ester compounds of the invention may be administered to treat leukemia and solid tumors in mammals, including humans. The esters of the present invention are prodrugs which are hydrolyzed to camptothecin compounds demonstrating inhibitory activity on topoisomerase I. The camptothecin compounds formed by hydrolysis of the esters of the invention are also effective in treating leukemia and solid tumors in mammals. Numerous camptothecin compounds have been shown to be effective against leukemia using the standard L1210 leukemia assay (Wall et al. (1993), Journal of Medicinal Chemistry, 36:2689-2700). High activity of camptothecin and camptothecin analogs has also been shown in the P388 leukemia assay (Wall (1983), Medical and Pediatric Oncology, 11:480A-489A). The later reference also provides a correlation between anti-leukemia activity as determined by the L1210 and the P388 leukemia assays with efficacy of camptothecin compounds against solid tumors. Compounds reported as active in the leukemia assays also have demonstrated activity in a number of solid tumors including a colon xenograft, a lung xenograft, a Walker sarcoma and a breast xenograft (Wall (1983), Table IV, page 484 A). Recent studies have confirmed the correlation between topoisomerase I inhibitory activity and anti-leukemia/anti-tumor activity of camptothecin compounds (Giovanella et al. (1989), Science, 246: 1046-1048). The compounds of the present invention are particularly effective in the treatment of colon, lung, breast and ovary solid tumors, brain glioma and leukemia. These compounds may also be used to treat maleria.

Having generally described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

1. 10,11-Methylenedioxy-Camptothecin-20-β-Ala-Lys-Ester Dihydrochloride

BOC-Lys (BOC)-β-ala-OH was prepared as reported in literature (Ref. R.L. Hudkins et al., *Bioorganic & Medicinal Chemistry Letters* **8** (1998) 1873-1876. A stirred solution of 10,11-MD-20(S)-CPT (785 mg; 20 mmol), BOC-Lys (BOC)-β-Ala-OH (1.0 g, 2.45 mmol), DMAP (80 mg), CH_2Cl_2 (400 mL) was treated with DCC (5.0 mL; 1.0 M solution of DCC in CH_2Cl_2). After 24 h, the reaction mixture was concentrated to 40 mL

and filtered. The filtrate was concentrated and purified by column chromatography (SiO_2, 100 g, $CHCl_3$). The BOC ester was obtained as light brown powder (1.09 g; 69%). ^{1}H-NMR (DMSO-d_6+D_2O) 0.93 (t, 3H), 1.10-3.56 (m, 33 H), 5.21 (s, 2H), 5.46 (s, 2H), 6.24 (s, 2H), 7.09 (s, 1H), 7.39 (s, 1H), 7.44 (s, 1H), 8.40 (s, 1H). MS m/z 791 (M^+).

The above CPT BOC-peptide ester (500 mg, 0.6 mmol) was dissolved in dry CH_2Cl_2 (70 mL) and the solution was cooled to 0°C. A solution of HCl (g) saturated-dioxane (3 mL) was added dropwise. After stirring for 2.5 h, the solvent was evaporated to give the crude salt. This material was taken in H_2O (75 mL) and extracted four times with $CHCl_3$ (4X40 mL). The aqueous solution was filtered and lyophilized to provide the product as an off-white solid (310 mg; 74%). The product was further purified by recrystallization from EtOH containing a few drops of 0.5 N HCl. ^{1}H-NMR (DMSO-d_6+D_2O)δ 0.92 (t, 3H), 1.15-1.67 (m, 5H), 1.47 (m, 2H), 1.66 (m, 2H), 2.18 (m, 2H), 2.60-3.12 (m, 4H), 5.22 (s, 2H), 5.48 (s, 2H), 6.19 (s, 2H), 7.15 (s, 1H), 7.39 (s, 1H), 7.49 (s, 1H), 8.44 (s, 1H).

2. **10,11-Ethylenedioxy-Camptothecin-20-β-Ala-Lys Ester Dihydrochloride**

The title compound was prepared as described in Example 1.

3. **7-Ethyl-10,11-MD-20(S)-CPT-20-β-Ala-Lys Ester Dihydrochloride**

The title compound was prepared as described in Example 1 using 7-ethyl-10,11-MD-20(S)-CPT as the starting material.

4. **Camptothecin-20-β-Ala-Lys Ester Dihydrochloride**

The title compound was prepared as described in Example 1 using Camptothecin as the starting material.

5. **7-Chloromethyl-10,11-MD-20(S)-CPT-20-β-Ala-Lys Ester Dihydrochloride**

The title compound was prepared as described in Example 1 using 7-chloromethyl-10,11-MD-20(S)-CPT as the starting material.

6. **10-Hydroxy-20(S)-CPT-20-β-Ala-Lys Ester Dihydrochloride**

The title compound was prepared as described in Example 1 using 10-benzyloxy camptothecin as the starting material.

7. **7-Ethyl-10-Hydroxy-20(S)-CPT-20-β-Ala-Lys Ester Dihydrochloride**

The title compound was prepared as described in Example 1 using 7-ethyl-10-benzyloxy-20(S)-CPT as the starting material.

8. 7-Ethyl-9-Amino-10,11-MD-20(S)-CPT-20-β-Ala-Lys Ester Dihydrochloride

A stirred solution of 7-ethyl-9-nitro-10,11-MD-20(S)-CPT (900 mg, 2.0 mmol), Boc-Lys (BOC)-β-Ala-OH (1.0 g, 2.45 mmol), DMAP (80 mg), CH_2Cl_2 (200 mL) was treated with DCC (5.0 mL); 1.0 M solution of DCC in CH_2Cl_2). After 24 h, the reaction mixture was concentrated to 30 mL and filtered. The filtrate was concentrated and purified by column chromatography (SiO_2, 100 g, $CHCl_3$). The BOC ester was obtained as an orange powder (1.33 g, 80%). ^{1}H-NMR (DMSO-d_6+$D_2$0)δ 0.92 (t, 3H), 1.03-3.40 (m, 38H), 5.31 (s, 2H), 5.50 (s, 2H), 6.47 (s, 2H), 7.18 (s, 1H), 7.68 (s, 1H). MS m/z 864 (M^+).

The above 7-ethyl-9-nitro-10,11-MD-20(S)-CPT-20-β-Ala-Boc-Lys ester (1.05 g, mmol) was dissolved in ethanol (120 mL). The catalyst (10% Pd/C, 150 mg) was added and the reaction mixture stirred under 1 atm of H_2 for 20 h. After removing the catalyst, the crude product was purified by chromatography (SiO_2, 100 g, $CHCl_3$; 1% MeOH/$CHCl_3$). Pure 7-ethyl-9-amino-10,11-MD-20(S)-CPT-20-β-Ala-BOC-Lys ester was obtained as a light orange powder (710 mg, 71%) ^{1}H-NMR (DMSO d_6+$D_2$0) δ0.92 (t, 3H), 1.05-3.83 (m, 38H), 5.25 (s, 2H), 5.54 (s, 2H), 6.20 (s, 2H), 7.02 (s, 1H), 7.08 (s, 1H). MS m/z 834 (M^+).

The above CPT-BOC peptide ester (500 mg, 0.58 mmol) was dissolved in dry CH_2CL_2 (50 mL) and the solution was cooled to 0°C. A saturated solution of HCl(g) in dioxane (3 mL) was added dropwise. After stirring for 2.5 h, the solvents were evaporated to half the volume. The precipitated product was collected and dissolved in $H_2$0 (70 mL). It was extracted four times with $CHCl_3$. The resulting aqueous solution was filtered and lyophilized to yield a tan-brown solid (290 mg, 71%). The product was further purified by recrystallization from EtOH containing a few drops of 0.5 N HCl. ^{1}H-NMR (DMSO-d_6+$D_2$0) δ0.95 (t, 3H), 1.32 (m, 2H), 1.37 (t, 3H), 1.47 (m, 2H), 1.66 (m, 2H), 2.11 (m, 2H), 2.69-2.85 (m, 4H), 5.32 (s, 2H), 5.57 (s, 2H), 6.21 (s, 2H), 7.02 (s, 1H), 7.22 (s, 1H).

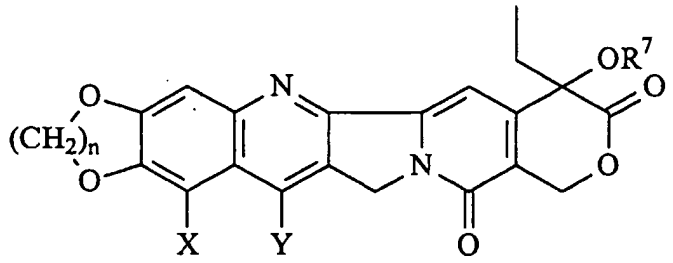
9. 9-Amino-10,11-MD-20(S)-CPT-20-β-Ala-Lys Ester Dihydrochloride

The title compound was prepared as described in Example 8 using 9-nitro-10,11-MD-20(S)-CPT as the starting material.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

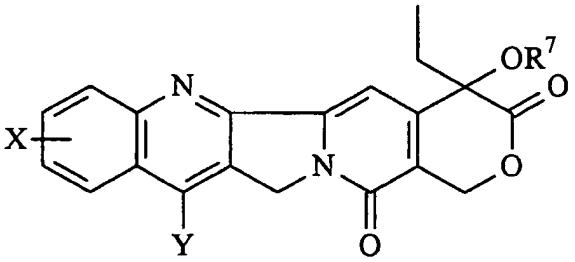
CLAIMS:

1. A camptothecin compounds having the structure:



(I)

or



(II)

where

X and Y are each independently NO_2, NH_2, H, F, Cl, Br, I, COOH, OH, O-C_{1-6} alkyl, SH, S-C_{1-6} alkyl, CN, NH-C_{1-6} alkyl, N(C_{1-6} alkyl)$_2$, CHO, C_{1-8} alkyl, N_3,

-Z-$(CH_2)_a$-N-$((CH_2)_bOH)_2$, wherein Z is selected from the group consisting of O, NH and S, and a and b are each independently an integer of 2 or 3,

-Z-$(CH_2)_a$-N-(C_{1-6} alkyl)$_2$ wherein Z is selected from the group consisting of O, NH and S, and a is an integer of 2 or 3,

-CH_2-L, where L is halogen (F, Cl, Br, I), $^+N_2$, $^+(OR^1)_2$, $^+S(R^1)_2$, $^+N(R^1)_3$, $OC(O)R^1$, OSO_2R^1, OSO_2CF_3, $OSO_2C_4F_9$, C_{1-6} alkyl-C(=O)-, C_{4-18} aryl-C(=O)-, C_{1-6} alkyl-SO_2-, perfluoro C_{1-6}alkyl-SO_2- and C_{4-18} aryl-SO_2-, (where each R^1 independently is C_{1-6} alkyl, C_{4-18} aryl or C_{4-18}ArC_{1-6}alkyl); or

-$CH_2NR^2R^3$, where (a) R^2 and R^3 are, independently, hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-6} alkyl, C_{2-6} alkenyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy-C_{1-6} COR^4 where R^4 is hydrogen, C_{1-6} alkyl, perhalo-C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-6} alkyl, C_{2-6} alkenyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkoxy-C_{1-6} alkyl, or (b) R^2 and R^3 taken together with the nitrogen atom to which they are attached form a saturated 3-7 membered heterocyclic ring which may contain a O, S or NR^5 group, where R^5 is hydrogen, C_{1-6} alkyl, perhalo-C_{1-6} alkyl, aryl, aryl substituted with one or more groups selected from the group consisting of C_{1-6} alkyl, halogen, nitro, amino, C_{1-6} alkylamino, perhalo-C_{1-6} alkyl, hydroxy-C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkoxy-C_{1-6} alkyl and -COR^6 where R^6 is hydrogen, C_{1-6} alkyl perhalo-

C_{1-6} alkyl, C_{1-6} alkoxy, aryl, and aryl substituted with one or more C_{1-6} alkyl, perhalo-C_{1-6} alkyl, hydroxy-C_{1-6} alkyl, or C_{1-6} alkoxy-C_{1-6} alkyl groups;

R^7 is C(O)-CH_2-CH_2-NR^8R^9, R^8 and R^9 are, independently, hydrogen, C_{1-8} alkyl, C(O)-$(CH_2)_m$-$NR^{10}R^{11}$, where m is an integer from 1 to 6, or -C(O)$CHR^{12}NR^{13}R^{14}$, where R^{12} is the side chain of one of the naturally occurring α-amino acids and R^{10}, R^{11}, R^{13} and R^{14} are each independently hydrogen or C_{1-8} alkyl; and

n is an integer of 1 or 2.

2. The camptothecin compound of claim 1, wherein n is 1.

3. The camptothecin compound of claim 1, wherein Y is -CH_2-L.

4. The camptothecin compound of claim 1, wherein L is selected from the group consisting of Cl, Br and I.

5. The camptothecin compound of claim 1, which is selected from the group consisting of 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-ethyl 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-chloromethyl 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-bromomethyl 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin; 7-hydroxymethyl-10, 11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin, 9-nitro 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin, 9-amino 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin, 7-ethyl-9-nitro 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin and 7-ethyl-9-amino 10,11-methylenedioxy-20-O-β-ala-lys-20-(S)-camptothecin;

10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-ethyl 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-chloromethyl 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-bromomethyl 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin; 7-hydroxymethyl-10, 11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin, 9-nitro 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin, 9-amino 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin, 7-ethyl-9-nitro 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin and 7-ethyl-9-amino 10,11-methylenedioxy-20-O-β-ala-20-(S)-camptothecin,

20-O-β-ala-lys-20-(S)-camptothecin; 7-ethyl-20-O-β-ala-lys-20-(S)-camptothecin; 7-chloromethyl-20-O-β-ala-lys-20-(S)-camptothecin; 7-bromomethyl-20-O-β-ala-lys-20-(S)-camptothecin;-20-O-β-ala-lys-20-(S)-camptothecin, 9-nitro-20-O-β-ala-lys-20-(S)-

camptothecin, 9-amino-20-O-β-ala-lys-20-(S)-camptothecin, 7-ethyl-9-nitro-20-O-β-ala-lys-20-(S)-camptothecin, 7-ethyl-9-amino-20-O-β-ala-lys-20-(S)-camptothecin,

20-O-β-ala-20-(S)-camptothecin; 7-ethyl-20-O-β-ala-20-(S)-camptothecin; 7-chloromethyl 20-O-β-ala-20-(S)-camptothecin; 7-bromomethyl-20-O-β-ala-20-(S)-camptothecin; 7-hydroxymethyl-20-O-β-ala-20-(S)-camptothecin, 9-nitro-20-O-β-ala-20-(S)-camptothecin, 9-amino-20-O-β-ala-20-(S)-camptothecin, 7-ethyl-9-nitro-20-O-β-ala-20-(S)-camptothecin and 7-ethyl-9-amino-20-O-β-ala-20-(S)-camptothecin.

6. A method of treating leukemia or solid tumors comprising administering to a patient in need thereof, the camptothecin compound of claim 1.

7. A pharmaceutical composition comprising the camptothecin compound of claim 1.

8. A method for inhibiting the enzyme topoisomerase I, comprising contacting a DNA-topoisomerase I complex with the camptothecin compound of claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/15033

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C07D 491/22; A61K 31/4741, 31/4745
US CL :546/41, 48; 514/279, 283
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 546/41, 48; 514/279, 283

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ON LINE:File Registry,File CAPLUS, File Beilstein, File Marpat (1907-2000).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,942,518 A (ASAHINA et al.) 24 August 1999, cols. 1-8.	1 and 3-7
Y	US 5,180,722 A (WALLl et al.) 19 January 1993, cols. 1-22.	1-8
A	US 5,856,333 A (CABRI et al.) 05 January 1999, see entire document.	6-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 JULY 2000	Date of mailing of the international search report 18 AUG 2000
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Form PCT/ISA/210 (second sheet) (July 1998)*